DTU Library

Development of a LC-QTOF screening method for pesticides and mycotoxins in cereals

Lykkeberg, Anne Kruse; Rasmussen, Peter Have; Poulsen, Mette Erecius

Publication date:
2014

Document Version
Publisher's PDF, also known as Version of record

DTU Food
National Food Institute

Development of a LC-QTOF screening method for pesticides and mycotoxins in cereals

Anne Kruse Lykkeberg, Peter Have Rasmussen and Mette Erecius Poulsen

DTU National Food Institute, Moerkhoej Bygade 19, DK-2860 Soeborg, Denmark; e-mail : alyk@food.dtu.dk

Introduction: The EURL for Pesticide residues in Cereals and Feeding stuff[1] has today a pesticide screening method using LC-QTOF (Agilent) with a database containing retention times of around 600 pesticides injected into the instrument. Other contaminants in cereals might also be relevant to include e.g. mycotoxins produced by fungi, which can arise at the field or during storage of cereals.

The aim of this project was to include relevant mycotoxins into the current pesticide screening method. In this study extraction and clean up of 25 mycotoxins and other secondary metabolites were investigated simultaneously together with selected pesticides. Recovery and matrix effect during the QuEChERS method were investigated.

1. Extraction
- Water
- Acetonitrile

2. Phase separation
- Magnesium sulphate
- NaCl, Citrate buffer

3. Freeze out step
- 1 h at -80 ºC
- Centrifugation

4. PSA clean up
- PSA
- Magnesium sulphate

Figure 1: Overview of the QuEChERS method. Samples were taken after the four steps.

Experimental: 25 mycotoxins at relevant concentration levels and 113 pesticides at 0.1 mg/kg were spiked into wheat flour (N=4). Spiked samples and blank samples were cleaned up using the QuEChERS method, and samples were taken after the 4 different steps specified in figure 1: After extraction with water and acetonitrile followed up with centrifugation (step 1), after phase separation with magnesium sulphate (step 2), after a freeze out step to remove lipids (step 3) and finally after PSA clean up (step 4). Matrix matched standards at the four steps using the blank samples and standards in solvent were prepared.
Recovery and Matrix-effect was calculated using the following equations:

$$Recovery = \frac{Area\ (spiked\ sample)}{Area\ (matrix\ standard)} \times 100\%$$

$$Matrix\ effect = \frac{Area\ (matrix\ standard)}{Area\ (pure\ standard)} \times 100\%$$

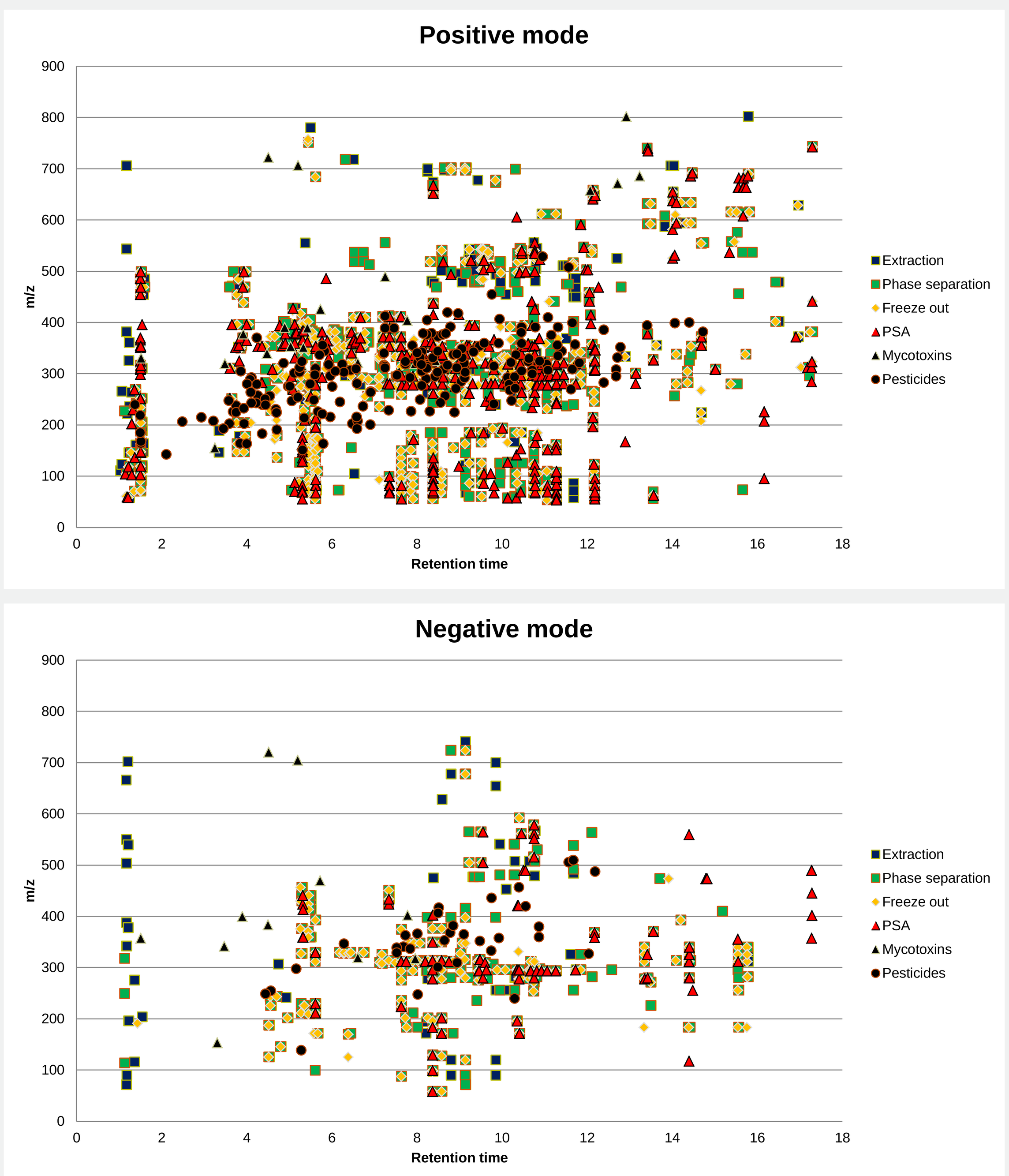

Figure 2 Matrix components in wheat samples after the 4 different clean up steps. Mycotoxins and pestcides are shown in black.

	Recovery				Matrix-effect			
	1	2	3	4	1	2	3	4
MYCOTOXINS								
Fumonisin B1	96	62	35	5	71	213	204	123
Fumonisin B2	76	73	36	6	153	166	151	111
Gliotoxin	134	86	102	48	52	72	72	88
HT2	90	117	116	106	196	55	58	95
Mycophenolic acid	111	101	107	8	223	130	139	79
Nivalenol	120	58	72	66	55	60	58	88
Ochratoxin A	105	94	103	ND	107	125	130	80
Patulin	126	76	98	116	182	307	242	161
Roquefortin C	153	101	92	92	31	38	41	75
PESTICIDES								
2.4-Dimethylformanilide	162	93	102	100	27	43	50	71
Acephate(I)	119	81	85	85	12	28	32	50
Acephate(II)	114	105	80	87	80	87	94	103
Aldicarb	101	94	74	106	24	20	26	41
Amitraz	103	57	72	63	62	49	67	90
Diazinon	118	160	151	111	29	19	24	60
Difenconazole	124	95	105	113	39	64	118	101
Epoxiconazole(I)	122	112	105	110	32	28	31	74
Ethoprop	126	108	108	100	28	22	25	69
Etofenprox	100	82	95	129	518	185	171	141
Fipronil	157	107	117	124	144	182	177	121
Isoprothiolane	199	104	104	109	18	30	70	97
Kresoxim-methyl	163	86	116	90	10	19	26	56
Malathion	186	121	135	86	12	10	16	61
Metaflumizone	164	98	114	133	187	157	157	108
Metconazole	174	111	107	133	37	54	102	96
Metribuzin	123	99	112	101	62	61	63	82
Oxadixyl	158	120	111	95	54	49	65	81
Oxamyl	85	88	88	106	370	239	169	104
Phenthoate	155	72	102	105	14	37	43	67
Phosmetoxon	162	109	118	108	58	66	71	87
Phoxim	170	58	115	150	28	76	103	62
Pirimiphos-methyl	171	115	116	73	21	26	42	62
Quinoxyfen	116	106	107	98	40	39	54	77
Tebufenozide	131	85	111	134	125	207	321	202
Triazophos	183	98	101	114	19	35	81	99
Trichlorfon	307	51	97	84	12	67	68	84
Trifloxystrobin	135	117	126	116	65	57	59	81
Zoxamide	113	83	97	96	21	24	42	77

Figure 3 Recovery and Matrix-effect of selected mycotoxins and pesticides. Most compounds showed acceptable recoveries at the step 2-4.

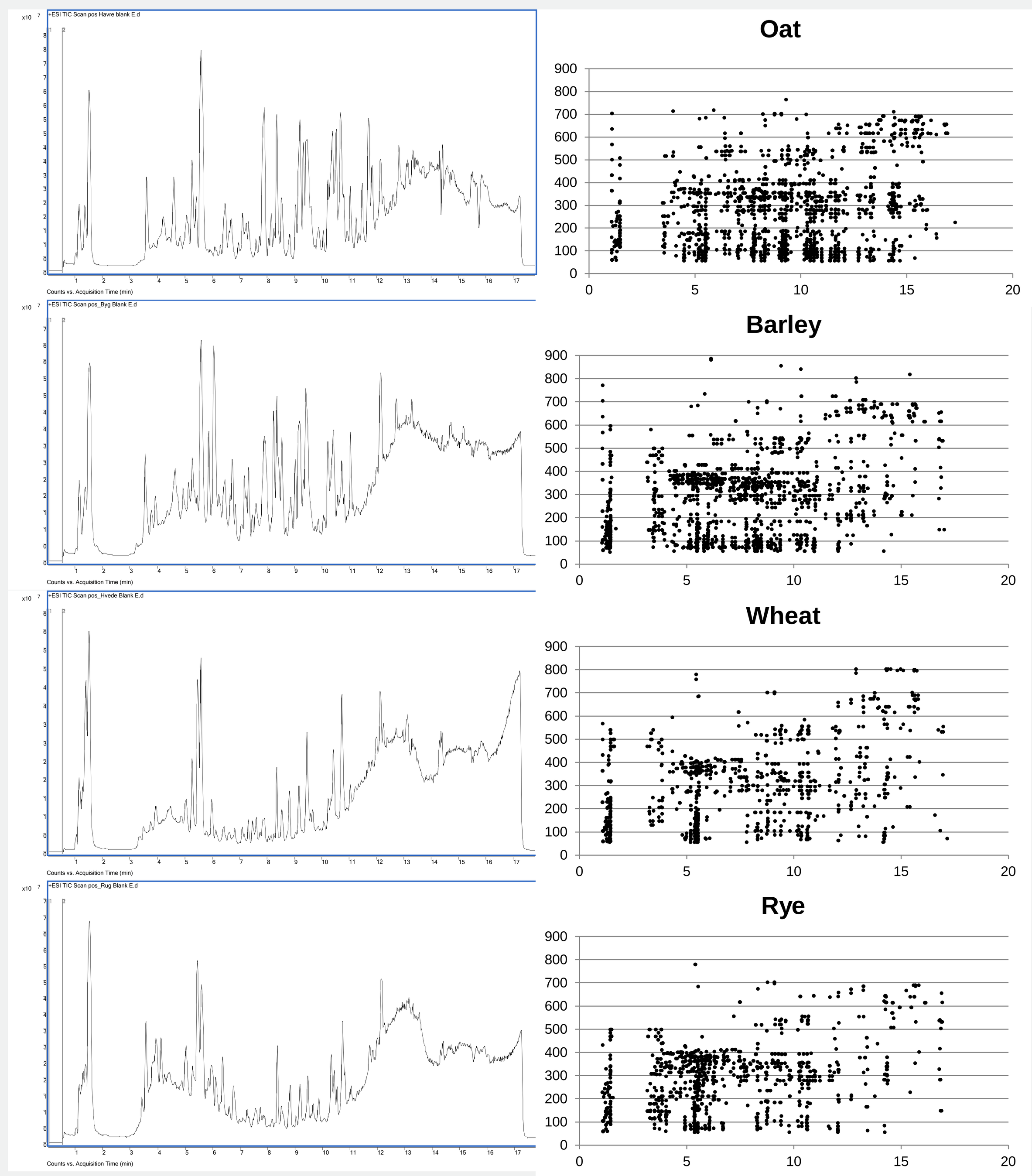

Figure 4 Total Ion Chromatograms and Matrix components of Oat, Barley, Wheat and Rye after the freezings step 3.

Discussions: The matrix components during the four steps are illustrated in Figure 2. It shows that more and more matrix-components are removed and the final PSA clean-up step 4 really removes significant amounts of matrix. Analytes are shown in black.
Recovery and matrix effect were similar in step 2-4 for most analytes, but some analytes had less matrix-effect after freezing and certainly after the PSA clean-up (Figure 3). However, several of the mycotoxins containing carboxylic acid groups are removed totally during the PSA step 3 (Figure 2). Recovery of the Fumonisins declines already after the freezing step 3. To choose the final sample preparation method a compromise is needed. Some matrix-effect is removed during the freezing step and therefore it was chosen to stop after step 3 before the PSA clean-up step 4, which removes acid analytes.
Matrix components and Total Ion Chromatograms of other cereals are shown in Figure 4. It is seen that Oat and Barley has more matrix components than wheat, and therefore more matrix-effect in these cereals are expected.

Conclusions: The QuEChERS method included a freezing step but without PSA clean up was chosen as the best compromise to analyse mycotoxins and pesticides in cereals.

[1] EURL-CF: EU Reference Laboratory for pesticide Residues in Cereals and Feeding stuff . DTU National Food Institute, Moerkhoej Bygade 19, DK-2860 Soeborg, Denmark
e-mail : eurl-cf@foood.dtu.dk, www.eurl-pesticides.eu